Origin and development of the vent tube in two species of the genus *Bufo*

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The vent tube of *Bufo arenarum* (medial configuration) and *Bufo fernandezae* (dextral configuration) tadpoles is derived from the ectodermal portion of the embryonic intestine exit. Ciliated epithelial cells provided by the epidermal surface (skin) stay as proctodeal mark inside of the cloaca during the larval development. The distal region of the embryo's proctodeum and larval epidermis contribute to form the definitive vent tube. The presumptive intestinal exit is present at Gosner stage 19, the anatomical cloaca is fully formed at stage 20 or early 21 (*urodaeum* and *proctodaeum*). After the common features are completed in *B. fernandezae*, the final configuration of the dextral vent tube becomes evident at stage 23 by the formation of a fold on the right side of the ventral fin. At stage 25, each type of vent tube takes its definitive shape and becomes functional. In both cases, a similar tissue organization of the cloaca and vent tube was found. For comparative purposes, the vent tubes of different types of larvae (1, 2 and 3 sensu ORTON, 1953) in stages 25-26, and with medial configuration, were examined at least, by one of the techniques indicated. They showed similar structural organization, among them and with the bufonids examined in this study; however, two layers of epithelial tissue separated by mesenchyme were always present.

INTRODUCTION

Tadpoles' vent tubes may have various configurations and may or not be associated with the anterior portion of the ventral fin. This tube usually projects from the medial part of the ventral body wall on the sagittal plane (ALTIG & JOHNSTON, 1989, MCDIARMID & ALTIG, 1999). Modifications of the distal parts of the ventral and/or right wall eventually produce a tube that opens either parallel with (medial) or to the right (dextral) of the plane of the ventral fin (ALTIG & MCDIARMID, 1999). The presence of such an extension for fecal transport is unique to anuran tadpoles. This larval feature develops during late embryonic stages, it has no adult derivates, and it atrophies at about the same time when the front legs emerge through the *operculum* (TAYLOR & KOLLROS, 1946, DEL CONTE & SIRLIN, 1951, GALLIEN & HOUILLON, 1951, VAN DIJK, 1959; ECHEVERRÍA, 1998).

Huettner (1948) described the cloaca of *Rana* as continuous with the *rectum*, and as formed partly by endodermic and ectodermic (*proctodaeum*, sensu Gadow, 1887, fide Van Dijk, 1959) tissues. Among bufonids, the formation of the proctodeal pit has been reported by several authors (Marchetti, 1919; Rondinini, 1928; Kagawa, 1932, Schechtman, 1939, for *B. bufo*, fide Van Dijk, 1959) without comments about its relationship with vent tube development.

In an attempt to supply the comparative morphological information required to evaluate the assumption that the presence of two vent tube configurations for the same function does not necessary mean different anatomical and/or histological features, I report on the ontogeny of the vent tube and the tissues that give rise to the vent tube.

Material and methods

A total of 100 embryos and tadpoles of *Bufo arenarum* and 20 of *Bufo fernandezae* were used to study the internal and external development of the vent tube between Gosner (1960) stages 17 and 25. Embryos were preserved in buffered 10 % formalin; *B. arenarum* embryos and larvae were fixed every 60 min. The specimens were examined with incident lighting and with a scanning electron microscope (SEM), after critical-point drying and gold-palladium coating. *B. fernandezae* specimens were reared in the laboratory for taking photographs in vivo of the main changes of the vent tube development.

For light microscopic examination, tadpoles and embryos were dehydrated intact in an alcohol series, embedded in paraffin (56-58°C), sectioned in transverse, sagittal and frontal sections of 4 or 6 μm, and stained with hematoxilin-eosin or Massons trichrome (Martoja & Martoja-Pierson, 1970). Histological terminology follows Welsh & Storch (1976). Pertinent drawings of the development were drawn with a camera lucida. For SEM observation, three tadpoles of *B. arenarum* (stages 27, 31 and 32) and four specimens of *B. fernandezae* (stages 23, 24, 25 and 26) were dissected to study the inner walls of the cloaca and vent tube.

The description of *B. fernandezae* vent tube development will be done in short form to explain differences from *B. arenarum*. For comparative purposes, I examined the vent tubes of tadpoles of different types (Orton, 1953) of larvae, in Gosner stages 25-26, of *Xenopus laevis* (Orton type 1), *Gastrophryne carolinensis* (Orton type 2), *Ascaphus truei* (Orton type 3), at least by one of the techniques described.

The term inner cloaca is used to indicate that region of the cloaca which is situated in the pleuroperitoneal cavity.

RESULTS

THE MEDIAL VENT TUBE OF *BUFO ARENARUM*

The first external evidence of the formation of the exit part of the gut appears at stage 16 as an indistinct pit on the posteroventral margin of the body. This pit is the incipient proctodeum. Ciliated epidermal cells occur in the wall of this depression (fig. 1a). Sagittal sections show an ectodermic invagination on the posterior part of the embryo There is no lumen in the hind-gut region (fig. 1b).

At stage 18, there is a definite depression although the proctodeum remains closed. All cells have yolk platelets, and cellular limits are not clear because of these platelets. In addition this epithelial cells have black pigment in the peripheral cytoplasm of their distal edges. Proctodeal cells appear more darkly pigmented, and these cells have long cilia that project into the lumen (fig. 1c). The cloaca *sensu stricto* is not yet formed. Opistonephric kidneys buds and pronephric ducts are not evident posteriorly, although the pronephric ducts have begun differentiation anteriorly.

By stage 19, this proctodeal pit is located slightly anterior to the beginning of the ventral fin anlage (fig. 1d) During stage 19, the cloacal membrane persists, that separates the intestine per se from the outside. Below the epidermis there are a few dispersed mesodermal cells. The proctodeum is separated from the endoderm by a layer of tissues hardy defined that constitute the "cloacal membrane" (ectoderm, endoderm, and poorly evident to absent mesenchyme) (fig. 1e). The proctodeum cavity is funnel-like, without connection to the inside of the gut, it is held by the dorsal part of the ventral fin anlage (fig 1f) Cells still have many yolk platelets that makes the delineation of individual cells difficult Black pigment remains in these cells.

At stage 20, the pronephric ducts finally connect to the distal part of the hind gut, and the functional larval cloaca is formed. A constriction in the cloaca marks the point of union between ectoderm and endoderm, and presence/absence of ciliated cells of the epidermis mark the limit between both tissue sources (fig 2a-b) The proctodeal epithelium is stratified, composed of two layers of cuboidal to polyhedral cells that rest on a basal layer.

At stage 21, an incipient peritoneum is forming in the pleuroperitoneal cavity Cellular surface specializations of the intestine are absent. A ventral constriction develops, that marks the posterior part of the ventral zone of the body (fig 2c) As the constriction grows inward, the vent cylindrical mass is more evident on the ventral side and separates from the body. The inner part of the larval cloaca has been composed by the *urodaeum* and part of the *proctodaeum*, before the ventral fin began to grow, and the tadpole's intestine became functional

By stage 22, the body and tail arc upwards slightly, and the vent opening occurs where the ventral fin contacts the body Mesenchymal cells grow between the tail muscles and the dorsal wall of the proctodeum The ventral fin grows at the expenses of the mesenchyme placed below the tail muscles, and in continuity with the posterior margin of the external orifice of the intestine (fig. 2d)

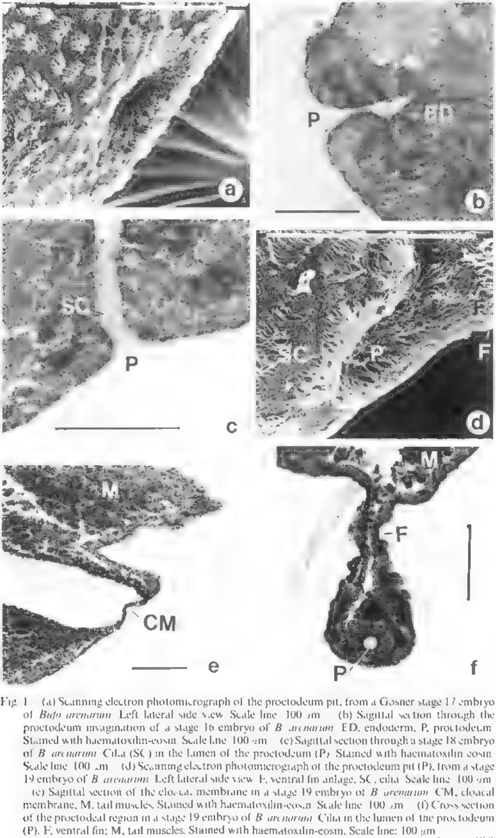

Fig. 1 (a) Scanning electron photomicrograph of the proctodeum pit, from a Gosner stage 17 embryo of *Bufo arenarum*. Left lateral side view. Scale line: 100 μm. (b) Sagittal section through the proctodeum invagination of a stage 16 embryo of *B. arenarum*. ED, endoderm; P, proctodeum. Stained with haematoxilin-eosin. Scale line: 100 μm. (c) Sagittal section through a stage 18 embryo of *B. arenarum*. Cilia (SC) in the lumen of the proctodeum (P). Stained with haematoxilin-eosin. Scale line: 100 μm. (d) Scanning electron photomicrograph of the proctodeum pit (P), from a stage 19 embryo of *B. arenarum*. Left lateral side view. F, ventral fin anlage; SC, cilia. Scale line: 100 μm. (e) Sagittal section of the cloacal membrane in a stage 19 embryo of *B. arenarum*. CM, cloacal membrane; M, tail muscles. Stained with haematoxilin-eosin. Scale line: 100 μm. (f) Cross section of the proctodeal region in a stage 19 embryo of *B. arenarum*. Cilia in the lumen of the proctodeum (P). F, ventral fin; M, tail muscles. Stained with haematoxilin-eosin. Scale line: 100 μm

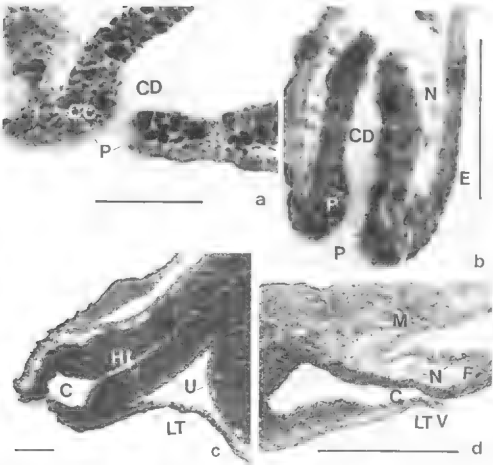

Fig. 2. (a) Sagittal section of cloacal region in a stage 20 embryo of *B. arenarum*. CD, endodermal cavity of the embryonic cloaca, CC, ciliated cells; P, proctodeum. Stained with Massons trichrome. Scale line: 100 µm. – (b) Cross section of the proctodeal region of a stage 20 embryo of *B. arenarum*. CD, endodermal cavity of the embryonic cloaca. E, epidermis, N, mesenchyme. P, proctodeum. Stained with Massons trichrome. Scale line: 100 µm. – (c) Sagittal section of the posterior intestine and cloaca of a stage 21 embryo of *B. arenarum*. C, cloaca, HI, posterior intestine, LT, ventral constriction, U, peritoneum trace. Stained with Massons trichromic. Scale line: 100 µm. – (d) Sagittal section of the inner cloaca (C) and vent tube anlage (V) of a stage 22 embryo of *B. arenarum*. F, ventral fin, LT, ventral constriction; M, tail muscles; N, mesenchyme. Stained with haematoxilin-eosin. Scale line: 100 µm.

At stage 23, the only external evidence of the vent tube anlage is a cylindrical mass of the epidermis that includes the presumptive hind limb buds (fig. 3a). Viewed externally, this mass is continuous with the skin of the body, and limb buds begin to separate from the vent structure at early stage 23. The aperture of the vent tube is slightly opened. At the end of this stage the limit between the body and the vent tube anlage is evident (fig. 3a). Ciliate cells are

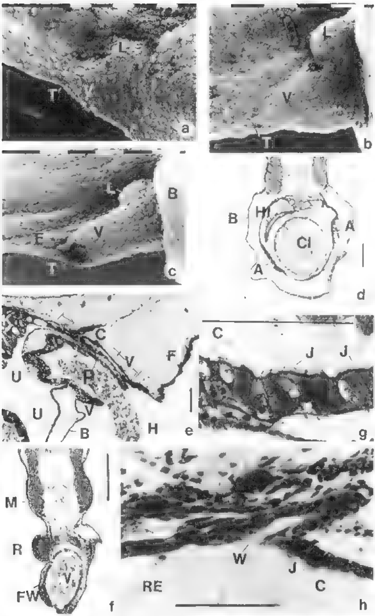

Fig. 3. (a) Scanning electron photomicrograph of the vent tube (V) and leg (L) buds in early stage 23 of *B. arenarum*. T, vent tube exit. Scale line: 100 μm. (b) Scanning electron photomicrograph of the vent tube (V) and the leg bud (L) in lateral view from stage 24 of *B. arenarum*. T, vent tube exit. Scale line: 100 μm. (c) Scanning electron photomicrograph of the vent tube (V) from a stage 25 of *B. arenarum*. B, body, F, ventral fin, L, leg bud, T, vent tube exit. Scale line: 100 μm. (d) Cross section of a stage 25 tadpole of *B. arenarum*. A, *rectus abdominis* muscle; B, body wall, CI, inner cloaca, HI, posterior intestine. Stained with Massons trichrome. Scale line: 100 μm. (e) Sagittal section of distal part of the cloaca (C) and vent tube (V) of a stage 26 tadpole of *B. arenarum*. B, body wall, F, ventral fin, H, fecal string, K, kidney, P, proctodeum zone, U, peritoneum. Stained with Massons trichrome. Scale line: 100 μm. (f) Cross section of a stage 26 tadpole of *B. arenarum*. FW, fin wall, M, tail muscles, R, right posterior limb, VT, vent tube. Stained with Massons trichrome. Scale line: 300 μm. (g) Sagittal section of the ciliated epithelium in the proctodeum zone of the cloaca (C) of a stage 26 *B. arenarum*. J, ciliated surface. Stained with Massons trichrome. Scale line: 50 μm. (h) Sagittal section of the dorsal wall of the distal part of the rectum (RE) next to the cloaca (C) of a stage 26 *B. arenarum*. J, ciliated surface, W, nephric duct orifice. Stained with Massons trichrome. Scale line: 50μm.

still present on the skin, the tail increases in length, and the ventral fin is well formed and attached to the posterior margin of the exit orifice (fig. 3a). The vent tube anlage grows in distal direction, forming its external tube, in expenses of the epithelial and mesenchymal cells around it.

At stage 24, the vent tube and limb buds are evident. The tube is conical, and the terminal aperture, which may be oval or circular, is open. The vent tube is still blocked inside (fig. 3b). At stage 25, the vent tube is fully developed and becomes functional. The aperture is circular and faces ventrally. The anterior edge of the ventral fin is connected to the posterior side of the vent tube (fig. 3c). Transversal section of the posterior region of the tadpole's body shows that the cloaca arises between the *rectus abdominis* muscles (fig 3d) They are attached to the posterior wall of the tadpole's body, and they are the only muscles next to the inner cloaca.

After its final organization in stage 25, the vent tube grows continuously. It grows during the later stages in expenses of the epidermis and dermis (fig. 3e) Epithelial and mesenchymal tissues of varying thickness are surrounding the vent tube cavity and form the ventral fin too (fig. 3f) The epidermis has definitively lost the cilia at stages after 25, but the surface of the proctodeum epithelia region has not (fig. 3g).

Several features in *B. arenarum* are important to be commented. The microvilli of the intestine appear at stage 24, in the anterior gut and midgut. The epithelium of the *rectum* is formed by a single layer of prismatic to cuboid cells with a low brushborder. Ciliated cells are only found behind the place where the nephric ducts enter the cloaca (fig 3h). The *proctodaeum* tissues do not make structural changes, at least during stages that have been covered in this study (17 to 32).

THE DEXTRAL VENT TUBE OF *BUFO FERNANDEZAE*

At stages 19 to 21, the embryo develops the cloacal exit presumptive zone and the dorsal and ventral fin buds (fig. 4a). At stage 22, in the posterior and ventral zone of the body, the vent tube anlage is evident (fig 4b). At stage 23, on the right side of the ventral fin next to the body appears a fold, which holds the incipient vent tube (fig 4c). At stages 24-25, the median and distal part of the vent tube is formed The tube takes it definitive shape and is functional at stage 25. The configuration is dextral and marginal to the ventral fin (fig. 4d).

Histological preparations of the vent tubes of *B. arenarum* and *B. fernandezae* showed that the last part of the alimentary tract (*rectum* and proximal part of the cloaca) runs on the left side of the pleuroperitoneal cavity, arising with medial configuration between the *rectus abdominis* muscles (fig. 3d, 5a). In both medial and dextral tubes, the proximal part of the vent tube is placed between both walls of the ventral fin (fig 3f, 5b-c); medial and distal parts of the vent tube of *B. fernandezae* are placed on the right wall of the fin, and are attached to its ventral edge (fig 5d-f).

The definitive vent tube in *B. arenarum* and *B. fernandezae* is composed of an inner layer of cuboidal epithelial tissue, surrounded by an outer wall (epidermis on most lateral and ventral sides) separated by mesenchyme of different thickness (fig 3e f, 5) This inner epithelium contains secretory cells, and is partially covered by ciliated cells on it proximal part, next to the inner cloaca

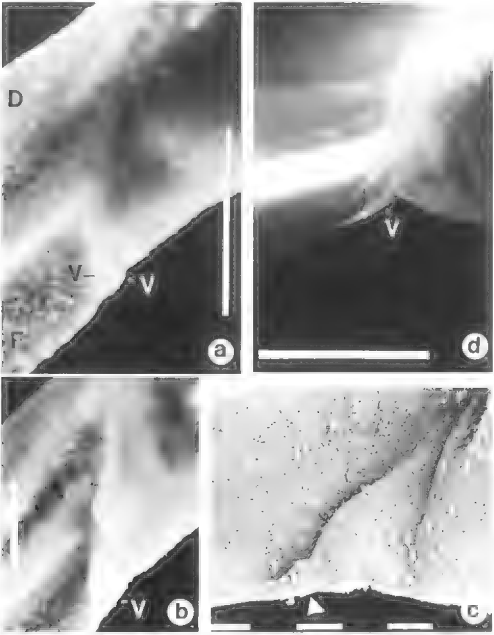

Fig. 4. – External morphology of the vent tube (V) of *Bufo fernandezae*. Photographs taken in vivo. – (a) Stage 21. Scale line = 1mm. – (b) Stage 23. Scale line = 1mm. – (c) Scanning electron microscope photomicrograph of the vent tube anlage (arrow). Stage 24. Scale line: 100 µm. – (d) Stage 24. Scale line = 1mm. – D, dorsal fin; F, ventral fin; V, vent tube.

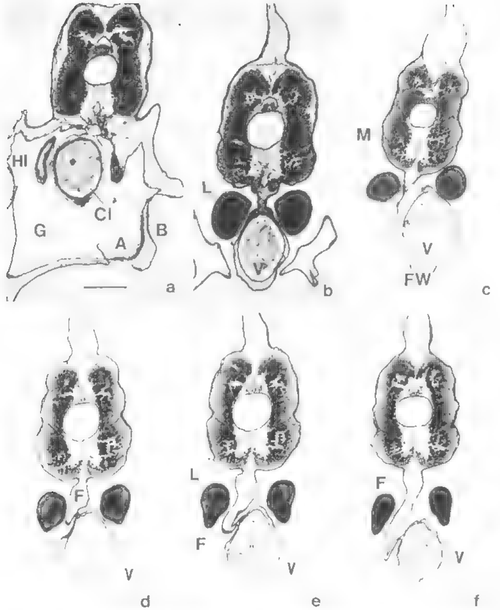

Fig. 5. Cross section of the posterior part of the body and tail of a tadpole of *B. fernandezae*. Stage 34. (a) Cloaca in the interior of the pleuroperitoneal cavity (G). (b-d) Median region of the vent tube. (e-f) Distal region of the vent tube. Scale line, 300 µm. A, *rectus abdominis* muscle; B, body wall; CI, inner cloaca; F, ventral fin; FW, fin wall; HI, posterior intestine; L, posterior leg buds; M, tail muscles; V, vent tube. Stained with Massons trichrome. Scale line: 300 µm

Cloacas of *B. arenarum* and *B. fernandezae* tadpoles dissected after the critical point procedure and observed by SEM confirmed that the ciliated cells are present in the distal portion of the inner cloaca and in the proximal part of the vent tube. Ciliated cells are absent on the distal wall of the vent tube after stage 26 (fig. 6a-c). In stage 25, microvilli of the surface of the intestines next to the cloaca are low and continuous (fig. 6c). In intestines full with food, the brushborder is more evident and dense than in the *rectum* and in the proximal part of the inner cloaca.

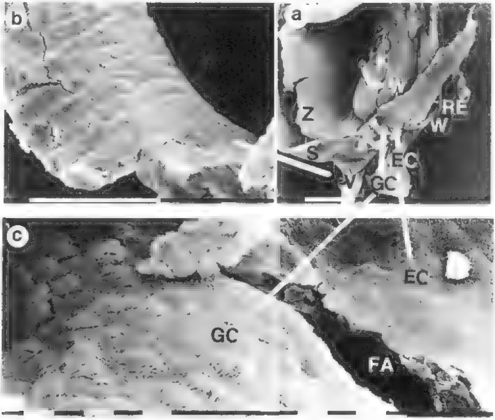

Fig. 6. – (a) Scanning electron microscope microphotograph of a dissected stage 32 tadpole of *B. arenarum*. EC, region of plane epithelium in the inner cloaca; GC, region with ciliated epithelium of the proctodeum; K, kidney; RE, hind intestine or rectum; S, skin; V, vent tube; W, nephric duct orifice; Z, parietal peritoneum. White scale line 1 mm. (b) Detail of the distal margin of the internal wall of vent tube with smooth surface in a stage 32 tadpole of *B. arenarum*. White scale line 0,1 mm. (c) Detail of the surface of the cloacal epithelium. EC, region of plane epithelium in the inner cloaca; FA, artifact; GC, region with ciliated epithelium of the proctodeum. White scale line 10 µm.

The larval cloaca of these tadpoles, could be "divided" in two anatomical parts, *urodaeum* and *proctodaeum*. Urinary bladders are not formed earlier than at stage 32. The region of the larval cloaca into which the nephric ducts open (*urodaeum*) is endodermal. The following region of the cloaca is ectodermal (*proctodaeum*) (fig. 3h).

Concerning the vent tube organization in the larvae of *Xenopus laevis* (type 1), *Gastrophryne carolinensis* (type 2) and *Ascaphus truei* (type 3), two layers of epithelial tissue separated by mesenchyme were always present The external layer corresponds to the skin of the ventral fin (stratified epithelium), the internal layer to the inner wall of the vent tube (simple cuboidal epithelium). The vent tube grows in centrifugal direction from the body wall backwards, and develops its definitive configuration.

DISCUSSION

From the results of this study, the term vent tube deserves to be analysed The posterior opening of the gut in anuran larvae was given several names, such as anus (HUETTNER, 1948), anal tube (INGER, 1985; CAMPBELL & CLARKE, 1998), anal opening (CEI, 1980), cloacal tube (ECHEVERRÍA & FIORITO DE LOPEZ, 1981), vent tube (ALTIG & JOHNSTON, 1986) or proctodeal tube (LAVILLA & LANGONE, 1991). The external tube is a structure formed from the epidermis as the larva grows. It is connected to the cloaca, and it could be considered a part of the cloaca of the embryo, specifically of the embryo proctodeum, an ectodermic structure. Vent tube can be interpreted, in two ways: (1) as a prolongation of the proctodeum at the expense of the ventral body skin, forming a tube from early stage 23 to the final 25, and (2) as an external tube formed just from the posterior edge of the body wall, specifically from the ventral and lateral epidermis that forms the ventral fin too This second view is based on the tissues from which the vent tube has started its development.

However, the external tube involves two kinds of epidermal tissues in the definite vent tube. original proctodeal material (parts of the inner wall) and epithelial tissues grown a posteriori (in inner and outer walls). MCDIARMID & ALTIG (1999) assumed the vent tube to be a prolongation of the abdominal skin This could be a wider interpretation enclosed in the second case The inner wall of the vent tube rises from the epithelial tissue attached to the margin of the distal cloacal area It is surrounded by the skin (epidermis and dermis) that forms the outer wall The connective tissue grows in width, and becomes more evident between both epithelia (fig. 7). On the basis of its anatomy, perhaps better names for the tube projected from the body wall for excreting feces could be at first sight proctodeal tube, cloacal tube or vent tube Proctodeal tube is restrictive only to a part of the cloaca, and it should not be considered as a prolongation of the original proctodeum because it has a different tissue composition. The term cloacal tube makes references to the functions and could be used in opposition to the functional connotation that anal tube has. The term vent tube is probably the best, it is a general term with no developmental connotation, unique to tadpoles, that could be better used by English speakers.

The vent tube is functional by stage 25 Its inner wall is partially covered by ciliated and secretory cells, that may probably contribute to the joining of feces into a string of intestinal

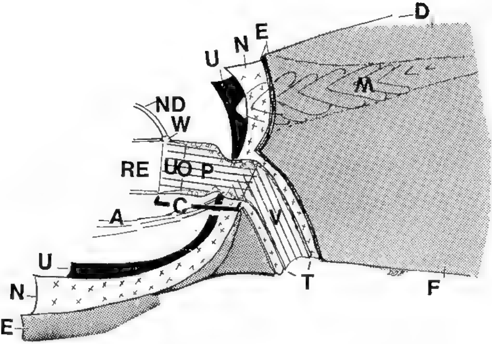

Fig. 7. – Schematic representation of a dissected cloaca (C) and vent tube (V) of a tadpole. A, *rectus abdominis* muscle; C, cloaca; D, dorsal fin; E, epidermis; F, ventral fin; M, tail muscles; N, loose connective (mesenchyme); ND, nephric duct; P, proctodeum; RE, hind intestine or rectum; T, vent tube exit; U, peritoneum; UO, urodaeum; V, vent tube; W, nephric duct orifice.

particles before they are excreted. No traces of muscles are found around the wall of the vent tube. The *rectus abdominis* muscle that is attached to the posterior wall of the body, next to the inner cloaca, has been studied by Carr & Altig (1992) in several tadpoles. These authors assume that this muscle could stabilize the abdomen and spiracular wall in suctorial tadpoles. Furthermore, contraction of this muscle could indirectly reduce the inner cloaca lumen, by compression. The compression over the lateral walls of the inner cloaca could help to remove part of the fecal string when it reaches the exterior.

Different cell specializations (brushborder and microvilli), formed after stage 24, are detected only in the intestines and are absent in the larval anatomical cloaca. Ciliated cells of the cloaca have their long cilia oriented to the posterior end of the body. They probably help to produce a current which conveys mucus and fecal particles through the cloaca, or they contribute to organize the fecal string before it leaves the vent tube.

No structural differences were found in the vent tube of bufonid tadpoles studied in this work. Intentionally, several tadpoles representing different anatomical and ecomorphological types, sensu Altig & Johnston (1989) (suctorial tadpole, *Ascaphus truei*, and suspension feeders, *Xenopus laevis*, *Gastrophryne carolinensis* and *Elachistocleis bicolor*), carrying medial vent tubes, were examined They showed similar structural organization based on epidermis and dermis. Probably this result suggests that vent tube configuration could have a taxonomic rather than ecomorphological significance.

RESUMEN

El tubo cloacal de las larvas de *Bufo arenarum* y *Bufo fernandezae* deriva de la porción ectodérmica de la abertura intestinal embrionaria Células epiteliales perduran como indicadoras del origen ectodérmico del proctodeo, permaneciendo en el interior de la cloaca durante todo el desarrollo larval. La región distal del proctodeo y de la epidermis larval contribuyen a formar el tubo cloacal definitivo. En el estadio 19 sólo está presente la abertura presuntiva del intestino; la cloaca larval se conforma en el estadio 20 o al inicio del 21. En el estadio 25, el tubo cloacal es funcional. Se examinaron con menor detalle especímenes de larvas con tubo cloacal medial de varios tipos (larvas tipo 1, 2 y 3) mostrando una organización tisular similar a los resultados obtenidos de la cloaca y del tubo cloacal de los bufónidos estudiados.

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